

## REMARKS

The invention relates to the identification and characterization of two classes of bone marrow stem cells: 1) small and rapidly self-renewing stem cells (RS cells), and 2) large, more mature marrow stromal cells (mMSC cells). The invention also relates to methods of their use.

Claims 1-10 have been cancelled and new claims 11-19 are newly presented. Therefore, claims 11-19 are currently under examination.

New claims 11-19 are fully supported in the as-filed specification. Support for these claims can be found, for instance, on p. 2, lines 17-18, p. 3, lines 20-23, p. 5, lines 12-15, p. 6, lines 9-12, 16-17 and 25-30, p. 7, lines 1-18 and 27-30, p. 8, lines 1-4, pp. 14-15, Table I, pp. 16-17, Table II and the Abstract. In particular, the data in Table I support the negative limitation of RS cells “not express(ing) STRO-1, PDGF-R, EGF-R, CD10 and CD147”.

Applicants appreciate the withdrawal of the rejection of Claim 9 under 35 U.S.C. §112, 2<sup>nd</sup> paragraph.

While the cancellation of claims 1-3 and 9 renders moot the rejections against them, the rejections are addressed below as they might apply to new claims 11-19.

### Rejection of claims pursuant to 35 U.S.C. §112, first paragraph – written description

The Examiner rejected claims 1-3 and 9, under 35 U.S.C. §112, first paragraph, as lacking written description. Specifically, the Examiner contended that: the terms “RS cell” and “mMSC” are not defined in the specification; the claims do not require the RS cells to express any of the recited polypeptides; the specification only discloses RS cells that express all four of the polypeptides recited in claim 1; and the claims encompass widely variant phenotypes.

Applicants respectfully submit that the specification provides ample written description for the invention as claimed, for the following reasons.

The invention relates to the discovery that mesenchymal (or marrow) stem cells (MSCs) actually comprise two sub-populations of cells that are physically and functionally different from each other. The two sub-populations are called 1) small and rapidly self-renewing stem cells (RS cells) and 2) large, more mature cells (mMSCs). The two sub-populations are distinguished from each other by size, morphology, and physical and functional properties, and are thereby defined. Specifically, RS cells are about 7 microns in diameter, comprise both

granular and agranular cells, have a rapid rate of replication and have enhanced potential for multilineage differentiation compared to mMSC cells. See, for instance, p. 3, lines 17-23, p. 3, line 29-p. 4, line 18, p. 5, lines 8-15, p. 6, line 24-p. 7, line 12 and the Figures. In contrast, mMSC cells are about 15-50 microns, divide more slowly, and have reduced potential for multilineage differentiation compared to RS cells. See, for instance, p. 3, lines 17-23, p. 5, lines 12-15, p. 6, line 24-p. 7, line 12 and the Figures. The two sub-populations of MSCs have been additionally characterized in the present application with regard to surface epitopes. This analysis revealed that mMSC cells express STRO-1, PDGF-R, EGF-R, CD10 and CD147, while RS cells do not. See Table I. While additional characterization of the sub-populations with regard to surface epitopes and protein expression is disclosed in Tables I and II, these disclosures are not necessary for distinguishing between the various MSC sub-populations.

The Court of Appeals for the Federal Circuit have reasoned that the factors to be considered in determining whether there is sufficient evidence of possession include the level of the skill and knowledge in the art, partial structure, physical and /or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406; MPEP 2163 II(A)(3).

The characteristics summarized above distinguish the two sub-populations. These characteristics are therefore sufficient to describe the two sub-populations of cells. The skilled artisan, presented with these disclosures, and the knowledge of the skilled artisan at the time the specification was filed, would thus understand that the inventor had possession of the claimed subject matter.

The Examiner further asserts that the specification “only discloses RS cells that express all 4 of [VEGF receptor-2 (FLK-1), TRK (an NGF receptor), transferrin receptor, and annexin II (lipocortin 2)].” The specification, however, describes two subtypes of RS cells, named RS-1 and RS-2. The two subtypes can be distinguished morphologically; RS-1 cells are agranular and RS-2 cells are granular. Applicants’ analysis of surface epitopes reveals additional distinguishing features between these two subtypes of RS cells. Specifically, Applicants found

that RS-1 cells express VEGF receptor-2 (FLK-1), TRK (an NGF receptor), transferrin receptor, and annexin II (lipocortin 2). See Table I. While some RS-2 cells express VEGF receptor-2 and TRK, RS-2 cells are distinguished from RS-1 cells in not expressing CD44, CD59, CD81 and CD90. See Table I. Thus, contrary to the Examiner's assertion, the specification does *not* "only disclose[s] RS cells that express all 4 of [VEGF receptor-2 (FLK-1), TRK (an NGF receptor), transferrin receptor, and annexin II (lipocortin 2)]."

Applicants respectfully submit that the extensive disclosure in the specification, as filed, provides ample written description for claims reciting a population of human marrow stromal cells enriched for RS cells, wherein about 95% of cells in the population are RS cells and wherein the RS cells are about seven microns in diameter, comprise granular and agranular cells, and do not express STRO-1, PDGF-R, EGF-R, CD10 and CD147 (see the specification on page p. 2, lines 17-18, p. 5, lines 12-15, p. 6, lines 16-17, p. 7, lines 13-18 and 27-30, p. 8, lines 1-4 and pp. 14-15, Table I). Therefore, there is ample written description in the specification as filed for claims relating to such a cell population. Claims 11-19 therefore satisfy the written description requirement of 35 U.S.C. §112, first paragraph.

#### Rejection of pursuant to 35 U.S.C. §112, first paragraph – enablement

Claims 1-3 and 9 were rejected pursuant to 35 U.S.C. §112, first paragraph, as lacking enablement for the full scope of the claimed invention. Specifically, the Examiner asserted that the specification is only enabling for a population of cells enriched for human small and rapidly self-renewing stem cells, wherein about 95% of the cells in said population are RS cells, and wherein said RS cells express all of VEGF receptor-2, TRK, transferring receptor and annexin II. The Examiner contends that the specification fails to disclose how to obtain and use the full scope of the claimed cell compositions.

As discussed above, the invention as claimed is drawn to a population of human marrow stromal cells named RS cells that are distinguished from other marrow stromal cells called mMSCs. The claimed invention is drawn to a population of human marrow stromal cells enriched for human small and rapidly self-renewing stem (RS) cells comprising about 95% RS cells, wherein the RS cells are about seven microns in diameter, comprise granular and agranular cells, and do not express any of STRO-1, PDGF-R, EGF-R, CD10 and CD147. Applicants have isolated this claimed population and described this in the specification. Therefore, the

specification has clearly taught how to obtain the claimed invention. Furthermore, Applicants' additional characterization of the various sub-populations of cells, with regard to the presence and absence of specific surface epitopes, enables the skilled artisan to obtain the claimed population using methods known in the art at the time of filing. For instance, the skilled artisan is familiar with FACS technology and the use of such technology to separate cell populations based on surface epitopes. The practice of such methods is routine in the art and should not be considered undue burden.

The Examiner further asserts that the specification does not disclose how to use the claimed cell compositions. Methods of using marrow stromal cells in cell and gene therapy are disclosed, for instance, on p. 5, lines 20-22. Furthermore, the skilled artisan would know how to use the claimed RS cells for research purposes, such as drug discovery relating to osteogenic, adipogenic, and/or chondrogenic differentiation. Therefore, there would not be undue experimentation for the skilled artisan to use the claimed cell population.

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. MPEP §2164.01 (citing *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976)). The fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. *Id.* Further, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. MPEP §2164.05(a) (citing *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991)). Therefore, under current law, enablement does not require a working example, and experimentation is allowed so long as it is not undue.

Accordingly, claims 11-19 are fully enabled under 35 U.S.C. §112, first paragraph.

#### Rejection of pursuant to 35 U.S.C. §102(b)

The Examiner rejected claims 1-3 under 35 U.S.C. § 102(b) as being anticipated by Bruder et al. (1997, J. of Cell. Biochem. 64:278-294). Specifically, the Examiner contended that the claimed composition is not directed to a composition that is more homogenous than that disclosed by Bruder et al. and, therefore, the teachings of Bruder et al. anticipate the claimed invention.

The claimed invention is drawn to a population of human marrow stromal cells enriched for human small and rapidly self-renewing stem (RS) cells comprising about 95% RS cells, wherein the RS cells are about seven microns in diameter, comprise granular and agranular cells, and do not express any of STRO-1, PDGF-R, EGF-R, CD10 and CD147.

It is hornbook law that “[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” MPEP §2131 (quoting *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). “The identical invention must be shown in as complete detail as is contained in the . . . claim.” *Id.* (quoting *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989) (emphasis added)). Therefore, Bruder must describe each and every element of the claims, in order to anticipate the claims under 35 U.S.C. §102(b). Bruder et al. fails to do so.

At best, Bruder et al. teaches a population of human marrow stromal cells and methods for culturing hMCSs. Therefore, Bruder does not disclose each and every element of the claimed invention. Bruder et al. does not teach or suggest a population of human marrow stromal cells enriched for cells that are about seven microns in diameter, comprise granular and agranular cells, and do not express any of STRO-1, PDGF-R, EGF-R, CD10 and CD147.

The Examiner rejected claims 1-3 pursuant to 35 U.S.C. §102(b) as being anticipated by Pittenger et al. (1999 Science 284:143-147). Pittenger discloses a population of human MSCs that are free of adult hematopoietic stem cells (HSCs). The flow cytometry data of Pittenger’s population of human MSCs clearly indicate that the population is different than the claimed population. For instance, Pittenger’s population expresses PDGF-R (Pittenger et al. 1999, Science, 284: Supplemental Figure 1, Table 2; copy attached hereto). Thus, Pittenger does not teach or suggest a population of human marrow stromal cells enriched for cells that are about seven microns in diameter, comprise granular and agranular cells, and do not express any of STRO-1, PDGF-R, EGF-R, CD10 and CD147.

Therefore, neither Bruder nor Pittenger anticipate the present invention because these references do not disclose each and every element of the claimed invention.

### Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application is now inapplicable, and that claims 11-19 are in condition for allowance. Applicants further submit that no new matter has been added by way of the present amendment. Consideration and allowance of these claims is respectfully requested at the earliest possible date.

Respectfully submitted,

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Enclosures: Petition for extension of time  
Pittenger et al. (1999 Science 284: Supplemental Figure 1. Table 2)





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ABSTRACT

### Multilineage Potential of Adult Human Mesenchymal Stem Cells

FULL TEXT

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## Supplementary Material

**Supplemental Figure 1.** Human mesenchymal stem cell (hMSC) isolation and characterization. (A) Karyotype analysis. The isolated hMSCs from three donors (2 male and 1 female) were tested at passage 12 by a commercial testing lab (Nichols Institute, San Juan Capistrano, CA). All donors were found to be normal. (B) Passaged hMSCs retain their telomerase activity. The telomeric repeat amplification protocol (TRAP-eze) (Oncor, Gaithersburg, MD) assay was performed on cultured human fibroblasts and mesenchymal stem cells (MSCs). Telomerase activity was revealed by the generation of radiolabeled TRAP products [ladder of bands starting at 50 base pairs (bp)] by cell lysates (+ lanes) and not by heat-inactivated lysates (- lanes). The dark band at the bottom of the non-denaturing polyacrylamide gel is an internal control (36 bp). Telomerase activity was detected in both early and late passage hMSCs.

**Isolation of hMSCs.** Informed consent was obtained from volunteer donors. hMSCs were isolated from marrow of the iliac crest of normal adults by a modification of the procedure of S. E. Haynesworth, J. Goshima, V. M. Goldberg, A. I. Caplan, *Bone* **13**, 81 (1992). About 20 to 30 ml of marrow aspirate was collected into a syringe containing 6000 units of heparin to prevent clotting. The marrow sample was washed with Dulbecco's phosphate-buffered saline (DPBS), cells were recovered after centrifugation at 900g, and the process was repeated once more. Up to  $2 \times 10^8$  nucleated cells at  $4 \times 10^7$  cells/ml were loaded onto 25 ml of Percoll of a density of 1.073 g/ml in a 50-ml conical tube. Cell separation was accomplished by centrifugation at 1100g for 30 min at 20°C. The nucleated cells were collected from the interface, diluted with two volumes of DPBS, and collected by centrifugation at 900g. The cells were resuspended, counted, and plated at 200,000 cells/cm<sup>2</sup>. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (low glucose) containing 10% fetal bovine serum (FBS) from selected lots. The serum lots selected for hMSC outgrowth from marrow aspirates were chosen for their ability to promote the growth of an adherent, well-spread colony-forming cell that, when placed into ceramic cubes and implanted into athymic mice, would give rise to bone and cartilage when evaluated histologically [D. P. Lennon *et al.*, *In Vitro Cell. Dev. Biol.* **32**, 602 (1996)]. The experiments described here utilized hMSCs that were grown in FBS lot AFE5185 from Hyclone (Logan, UT). Medium was replaced at 24 and 72 hours and every third or fourth day thereafter. hMSCs grew as symmetric colonies and were subcultured at 10 to 14 days by treatment with 0.05% trypsin and 0.53 mM EDTA for 5 min, rinsed from the substrate with serum-containing medium, collected by centrifugation at 800g for 5 min, and seeded into fresh flasks at 5000 to 6000 cells/cm<sup>2</sup>. With each treatment of trypsin-EDTA and replating, the passage number was increased and represented approximately three population doublings.

**Supplemental Figure 1. Table 1.**

Average volume of marrow aspirate	10 ml
Number of nucleated cells	280 x 10 <sup>6</sup> /ml
Average number of cells after density centrifugation	~100 x 10 <sup>6</sup> (30%)
Cells plated at 200,000/cm <sup>2</sup>	3 flasks (185 cm <sup>2</sup> size)
Average number of cells at passage 0 Replate cells at 1 x 10 <sup>6</sup> /flask	2 million to 5 million/flask = 6 million to 15 million
Average number of cells at passage 1 Replate cells at 1 x 10 <sup>6</sup> /flask	3 million to 5 million/flask = 18 million to 75 million
Average number of cells at passage 2	3 million to 5 million/flask = 54 million to 375 million

**Flow cytometry.** The cells were harvested from the tissue culture flasks by treatment with 0.05% trypsin or 25 mM EDTA in phosphate-buffered saline (PBS). The cells, in solution at a concentration of 0.5 x 10<sup>6</sup> cells/ml, were stained for 20 min with an empirically determined amount of each antibody, generally 10 to 20 µl. Labeled cells were thoroughly washed with two volumes of PBS and fixed in flow buffer (1% paraformaldehyde, 0.1% sodium azide, and 0.5% bovine serum albumin in PBS). The labeled cells were analyzed on a FACScan or FACSVantage (Becton-Dickinson) by collecting 10,000 events with the Cell Quest software program (Becton-Dickinson).

**Supplemental Figure 1. Table 2. Flow cytometry characterization of hMSCs**

Integrins - Positive		Integrins - Negative	
α1	CD49a	α4	CD49d
α2	CD49b	αL	CD11a
α3	CD49c	Cβ2	CD18
αα	CD49e	Hematopoietic Markers -Negative	
αv	CD51	T4	CD4
β1	CD29	Mo2	CD14
β3	CD61		CD34
β4	CD104	Leukocyte Antigen	
			CD45
Cytokine Receptors - Positive		Cytokine Receptors - Negative	
IL-1R	CD121a	IL-2R	CD25



IL-3R $\alpha$       CD123

IL-4R      CDw124

IL-6R      CD126

IL-7R      CDw127

Factor Receptors - Positive

Factor Receptors - Negative

IFN $\gamma$ R      CDw119

EGFR-3

TNFIR      CD120a

TNFIIR      CD120b

TGF $\beta$ IR

TGF $\beta$ IIR

Other - Negative

bFGFR

Fas ligand

PDGFR      CD140a

T4

CD4

Transferrin      CD71

Matrix Receptors Positive

Matrix Receptors - Negative

ICAM-1      CD54

ICAM-3      CD50

ICAM-2      CD102

E-Selectin      CD62E

VCAM-1      CD106

P-Selectin      CD62P

L-Selectin      CD62L

PECAM-1      CD31

LFA-3      CD58

vW Factor

ALCAM      CD166

Cadherin 5

Hyaluronate      CD44

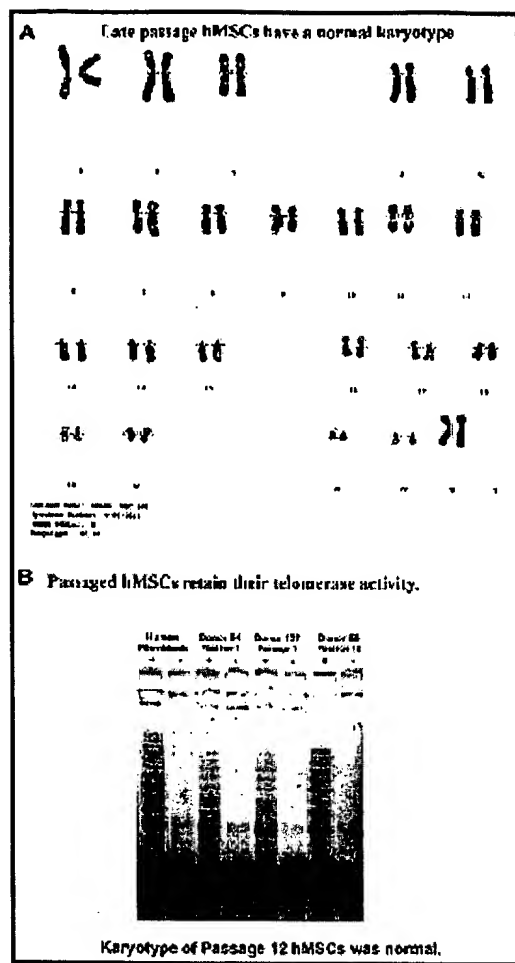
Lewis<sup>x</sup>      CD15

Endoglin      CD105

Others - Positive

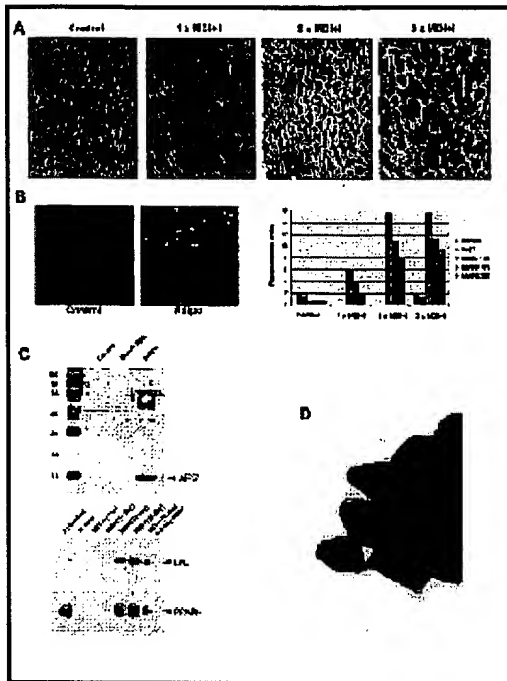
CD9

Thy-1      CD90

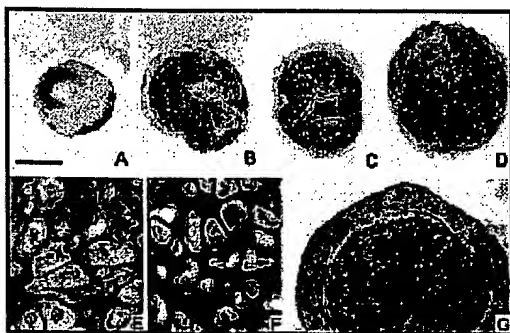


**Supplemental Figure 2.** Adipogenic differentiation of marrow-derived stem cells. (A) hMSCs were cultured as monolayers in DMEM containing 10% FBS and antibiotics and allowed to become confluent. The cells were cultured for an additional 3 to 7 days and then adipogenic induction (MDI+I) medium containing 1  $\mu$ M dexamethasone and 0.5 mM methyl-isobutylxanthine, insulin (10 (g/ml), 100 mM indomethacin, and 10% FBS in DMEM was added. The hMSCs were incubated in this media for 48 to 72 hours, and the media was changed to adipogenic maintenance (AM) medium containing insulin (10  $\mu$ g/ml) and 10% FBS in DMEM for 24 hours. Lipid vacuoles were first detectable within the cells at 48 hours of the first MDI+I treatment. The cells were then re-treated with MDI+I for a second or third treatment. The cultures were then maintained in AM for 1 week before fixation. Adipogenic differentiation was demonstrated by the accumulation of lipid vesicles and by the expression of adipose-specific genes. Multiple treatments resulted in increasing numbers of adipocytes, as shown by oil red O staining (A). (B) A fluorescence assay based on Nile red staining in the lipid vacuoles and normalization relative to 4',6'-diamidino-2-phenylindole staining of DNA demonstrated the 9- to 16-fold increase over control cultures at 2 weeks. (C) Adipose-associated gene products were shown by immunoblotting, using an antibody to aP2 or using a cDNA probe for lipoprotein lipase (LPL) or peroxisome proliferator-activated receptor  $\gamma$ 2 (PPAR $\gamma$ 2) [Murine cDNA probes for LPL and PPAR $\gamma$ 2 were the gift of J. Gimble (University of Oklahoma, School of Medicine, Oklahoma City), and polyclonal antibody to aP2 was provided by M. D. Lane (Johns Hopkins University, School of Medicine, Baltimore, MD).] Immunoblotting analysis of extracts of the adipogenic cells showed high levels of expression of aP2 [(C), adipogenesis (Adipo) lane], but undifferentiated or nonadipogenic cells that were present in the same culture [such as those present in 2 x MDI+I in (A)] failed to express detectable aP2 (control and non-Adipo lanes). LPL was present as two mRNAs of 3300 and 3700 nucleotides (nt), and PPAR $\gamma$  was present as a single mRNA of ~1800 nt. Northern (RNA) blots of extracts of the adipogenic cells showed high-level expression of PPAR $\gamma$  and LPL but showed no expression in the undifferentiated cultured

mesenchymal cells [(C), lower panels]. (D) Lipid vesicles in adipocytes coalesced over 3 months in culture to form one or two large lipid inclusions.

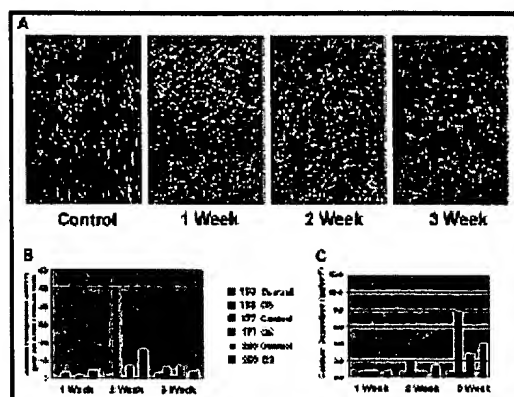


**Supplemental Figure 3.** Chondrogenic differentiation of marrow-derived stem cells. Cultured marrow-derived stem cells were grown as a pelleted micromass with the inclusion of TGF $\beta$ 3. Under low-speed centrifugation, a dense mass of cells formed at the bottom of the conical centrifuge tube. The cells consolidated within 1 day to a single mass that could be dislodged to float freely in suspension culture. Safranin O staining for proteoglycans revealed an increasing amount over the 3-week time period associated with the morphological differentiation to a chondrocyte phenotype: (A) 7 days, (B) 14 days, (C) 21 days, and (D) 28 days. The differentiated cells were also highly reactive with C4F6 monoclonal antibody to type II collagen. The chondrogenic cells could be induced to further differentiate to hypertrophic chondrocytes that expressed (E) type X collagen (monoclonal X53 from Quartett, Berlin) as well as (F) type II collagen (monoclonal C4F6). The chondrogenic cells also expressed (G) the extracellular matrix proteoglycan aggrecan (polyclonal antiaggrecan from D. Heinegard, Lund University, Lund, Sweden). The cell pellet increased in size over a 2- to 3-week period, and this is due almost entirely to the deposition of extracellular matrix rather than continued cell division [A. M. Mackay, S. C. Beck, J. M. Murphy, F. P. Barry, M. F. Pittenger, *Tissue Eng.* 4, 472 (1998)].



**Supplemental Figure 4.** Osteogenic differentiation of marrow-derived stem cells. (A) Osteogenic differentiation of marrow-derived stromal cells over a period of 21 days was demonstrated by the

increase in alkaline phosphatase and the accumulation of calcium, which has been shown to be in the form of crystalline hydroxyapatite [N. Jaiswal, S. E. Haynesworth, A. I. Caplan, S. P. Bruder, *J. Cell. Biochem.* **64**, 295 (1997)]. Alkaline phosphatase was detected histologically (Sigma kit 85), and the mineral was stained with silver by the method of von Kossa. (B) The quantification of alkaline phosphatase demonstrated an increase in activity between 7 and 14 days that then subsided, whereas (C) that for calcium indicated that the accumulation continued to at least 3 weeks.



**Supplemental Figure 5.** Differentiation is restricted to the directed lineage as shown by reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR was used to analyze the expression of lineage-related mRNAs and to reveal whether any cells differentiated to the unintended lineages. hMSCs were cultured in each differentiation condition for up to 2 weeks, and RNA was then isolated. At this time, most of the cells had differentiated to the desired lineage, and the RT-PCR reaction was performed and the resultant cDNA population was tested for expression of PPAR $\gamma$ , aP2, type II collagen, type IX collagen, osteopontin, and alkaline phosphatase (AP). Primers for  $\beta$ 2 microglobulin (B2M) were used as a control to assure even loading of the gel. Cells were rinsed in DPBS, lysed in RLT lysis buffer (Qiagen, Santa Clarita, CA), total RNA was purified with Qiagen RNeasy spin columns according to the manufacturer's recommendations, and the isolated RNA was quantified by ultraviolet spectroscopy. For PCR analysis, 1  $\mu$ g of RNA was converted to cDNA with Moloney murine leukemia virus RT and random hexamer primers. The reagents were purchased from Perkin-Elmer (Foster City, CA), and experiments were performed with a Perkin-Elmer GeneAmp 9600 PCR system and MicroAmp reaction tubes. The PCR reactions were carried out for 30 cycles with the primers given below. The reaction products were resolved by electrophoresis on a 1.5% agarose gel and visualized with ethidium bromide. PCR primers were as follows:

PPAR $\gamma$ 2 (352 bp product)

5'-GCTGTTATGGGTGAACTCTG.....CTCGGACGTAGAGGTGGAATA-3'

Fatty acid binding protein aP2 (114-bp product)

5'-TGGTTGATTTTCCATCCCAT.....CAGTTTAAGGACCGGGTCAT-3'

Type II collagen 1 $\alpha$  (377-bp product)

5'-TTTCCCAGGTCAAGATGGTC...ACCACTCTGTCCACGACTTC-3'

Type IX collagen  $\alpha$ 1 (159-bp product)

5'-GTGTTGCTGGTGAAAAGGGT.....TAATCCTGGTCACCCTAGGG-3'

Osteopontin (330-bp product)

5'-CTAGGCATCACCTGTGCCATACC....CTACTTAGACTACTTGACCAGTGAC-3'

Alkaline Phosphatase (453 bp product) 5'-

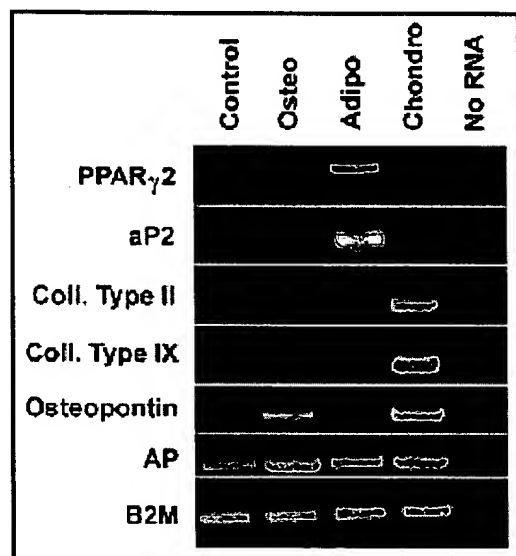
TGGAGCTTCAGAAGCTCAACACCA...CCTGACCATGAGTCTGTTGCTCTA-3'

$\beta$ 2 Microglobulin (270-bp product)

5'-CTCGCGCTACTCTCTCTTTCTGG..GCTTACATGTCTCGATCCCACTTAA-3'.

PPAR $\gamma$ 2 and aP2 were only expressed at detectable levels in the adipogenic cultures, whereas type II and type IX collagens were only found in the chondrogenic conditions. Osteopontin was expressed in the

osteogenic and chondrogenic pathways, with low-level expression in the undifferentiated hMSCs (Control). Alkaline phosphatase was expressed in all preparations, but was highest in the osteogenic hMSCs. Peroxisome proliferator-activated receptor (PPAR)  $\gamma$ 2 and the fatty acid binding protein aP2 were only expressed in the adipogenic cultures, whereas type II and type IX collagens were only expressed in the chondrogenic cultures. Osteopontin was found in chondrogenic as well as in osteogenic cultures, which is consistent with in vitro differentiation of these tissues. Alkaline phosphatase was present in all cultures but was most elevated in the osteogenic cultures. Cbfa-1 expression, as tested by RT-PCR, was not a good marker for osteogenesis, as it was expressed in the undifferentiated cells and expression persisted in cells differentiated to the other lineages (R. K. Jaiswal and M. F. Pittenger, data not shown).  $\beta$ -2-microglobulin was used as a control for equal loading in these experiments. Additional information on hMSCs is available at <http://www.osiristx.com>.



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